

WHAT IS CLAIMED IS:

1. A polypeptide fragment of an HCV helicase protein, which is derived from a subdomain of the HCV NS3 helicase protein, wherein the fragment is less than 30 kDa, 5 structurally sound, soluble, monodisperse, and stable in a buffered solution.
2. A polypeptide fragment of claim 1, which is suitable for use in nuclear magnetic resonance.
- 10 3. A polypeptide fragment of claim 1, wherein the subdomain comprises amino acids 181 to 324 of the HCV helicase protein.
4. A polypeptide fragment of claim 3, which comprises a single amino acid substitution, the amino acid selected from Asp 249 and Arg 257, wherein the substitution is a nonpolar 15 amino acid.
5. A polypeptide fragment of claim 4, wherein the substitution for Asp 249 is lysine or arginine, and wherein the substitution for Arg 257 is glutamic acid or aspartic acid.
- 20 6. A polypeptide fragment of claim 1, wherein the subdomain comprises amino acids 327 to 481 of the HCV helicase protein, and wherein the amino acid residues at positions 431 to 451 are deleted and replaced by an amino acid sequence selected from SEQ ID NOS: 2, 7, 8, 9, 10, 11, 12, 13, and 14.
- 25 7. A polypeptide fragment of claim 1, wherein the subdomain comprises amino acids 181 to 481 of the HCV helicase protein, and wherein the amino acid residues at positions 431 to 451 are deleted and replaced by an amino acid sequence selected from SEQ ID NOS: 2, 7, 8, 9, 10, 11, 12, 13, and 14.

8. A polypeptide fragment of claim 1, wherein the subdomain comprises amino acids 181 to 572 of the HCV helicase protein, and wherein the amino acid residues at positions 328 through 482 are deleted.

5 9. A polypeptide which comprises an amino acid sequence selected from SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide comprising SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:6 optionally comprises a single amino acid substitution selected from Asp 249 and Arg 257, wherein the substitution is a nonpolar amino acid.

10

10. A polypeptide of claim 1, which is in a complex with a small molecule HCV helicase inhibitor.

15

11. A crystalline composition, comprising a polypeptide fragment derived from subdomain I.

12. The crystalline composition of claim 11, wherein the polypeptide fragment further comprises a single amino acid substitution, the amino acid selected from Asp 249 and Arg 257, wherein the substitution is a nonpolar amino acid.

20

13. The crystalline composition of claim 12, wherein the substitution for Asp 249 is lysine or arginine, and wherein the substitution for Arg 257 is glutamic acid or aspartic acid.

25

14. The crystalline composition of claim 12, wherein the polypeptide fragment comprises an amino acid sequence of SEQ ID NO: 3.

15. A crystalline composition comprising the structural coordinates set forth in Table 5.

16. A precipitant solution comprising from 1 to 60 µg of an HCV helicase fragment, from 30 5 to 35% weight to volume of a precipitant compound, from 1 to 1000 mM of a salt, a buffer

for a precipitant solution and optionally a protein stabilizing agent, wherein the pH of the solution is from about 4 to 7 and the temperature is from about 1 to 26°C.

17. A buffered solution comprising from 50 to 1000 μ M of an HCV helicase fragment,

5 from 5 to 15% weight to volume of D₂O, a protease inhibitor, 25 to 250 mM KPO₄, optionally about 25 to 250 mM sodium chloride, optionally about 0.010 to 0.020% sodium azide and 1 to 10 mM DTT, wherein the pH of the solution is from about 4 to 8.

18. A method for preparing a purified crystalline composition which comprises a purified

10 polypeptide fragment of claim 1, comprising the steps of:

stabilizing the purified polypeptide fragment in a solution comprising a protein stabilizing agent;

purifying the polypeptide from the solution by applying the solution to an anion exchange chromatography column and collecting the fraction containing the polypeptide; and

15 crystallizing the polypeptide in the fraction by allowing crystals to form a precipitant which comprises a protein stabilizing agent, a salt and a precipitant compound under conditions which permit crystallization.

19. The method of claim 18, wherein the polypeptide fragment comprises an amino acid

20 sequence of SEQ ID NO: 3.

20. A method for identifying an inhibitor compound of an HCV helicase protein,

comprising the steps of:

obtaining a helicase polypeptide fragment which is contacted with a compound, 25 wherein the fragment comprises at least subdomain I or subdomain II of the HCV NS3 helicase protein;

assaying the fragment which is contacted with the compound and assaying the HCV helicase protein for activity;

and comparing the activity of the fragment in contact with the compound to the 30 activity of the HCV helicase protein, wherein a decrease in the activity of the fragment

compared with the HCV helicase protein identifies the compound as an inhibitor of HCV helicase activity.